



Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Recovery of marine *Conus* (Mollusca: Caenogastropoda) from imposex at Rottneest Island, Western Australia, over a quarter of a century

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ARTICLE INFO

Keywords:

Tributyltin
TBT
Thais
Nucella

ABSTRACT

Imposex is a reproductive abnormality in which female snails begin to transform to males, but do not become functional. It was caused by tributyltin (TBT) used as an antifoulant in boat paints. Imposex was first recorded marine snails (*Conus*) (Mollusca: Caenogastropoda) at Rottneest Island, Western Australia, in January 1991, where 88% of individuals at the west end were affected. Most were at moderate Stages 3 and 4 on a scale of 0 (no affect) to 6 (death). TBT was banned on boats < 25 m long in late 1991 in WA. In 1996, imposex had declined to 69% of females with Stages 3 and 4 still the most common. By 2007 only 35% of females exhibited imposex; Stage 3 was the highest level recorded. TBT was below detection limits. TBT was banned on vessels > 25 m in September 2013. In February 2017 only 4% of *Conus* had imposex, at Stage 1.

1. Introduction

Tributyltin (TBT) was widely used as an antifoulant additive to boat paints starting in the late 1960s. TBT was an extremely powerful biocide that economically and significantly reduced biofouling on the hulls of vessels. After application, the TBT gradually leached out, preventing organisms from attaching to the hull. Unfortunately, TBT was then free in the water column and still retained its lethal effects. Once TBT was widely used there was rapidly increasing evidence of unexpected and serious side effects, even at concentrations as low as a 1 µg per litre (one part per trillion). Thirty years ago, Goldberg (1986) described TBT as “the most toxic substance ever deliberately introduced into natural waters”. TBT had a wide range of effects on plants and animals. As with many other chemicals, TBT was concentrated as it moved progressively higher in food chains. Effects have been demonstrated in a variety of fish and mammals at or near the tops of foodwebs (Suzuki et al., 1992; Kim et al., 1996; Tanabe, 1999).

The best-known effect of TBT is imposex, which occurs in dioecious species of marine gastropods, particularly in the family Muricidae. Males are apparently unaffected but there is a progressive masculinisation of females; the higher the TBT concentration and/or the longer the exposure, the greater the effect. Females begin to develop a rudimentary penis and/or a vas deferens leading from the penis to the

gonad at concentrations as low as one part per trillion. Females never become functional males, but in extreme cases the female gonopore becomes sealed over and she is unable to expel gametes. These continue to accumulate until the gonoduct breaks and the female dies. Even if the females do not die, the population can suffer severe losses if they are unable to reproduce (Gibbs et al., 1987).

TBT is not the only cause of imposex. Copper, paint matrix, and environmental stresses can all cause imposex (Nias et al., 1993). Evans et al. (2000) demonstrated that injection of nonylphenol, an estrogen mimic, could cause imposex in the European muricid gastropod *Nucella lapillus* (Linnaeus, 1758). In addition, imposex can occur ‘naturally’ in low concentrations. For example, excreta from gull colonies may cause low levels of imposex in *N. lapillus* (Nias et al., 1993). However, TBT has been widely used and is the cause of most reported cases of imposex.

Imposex attributed to TBT was first reported by Blaber (1970) in *N. lapillus* in the United Kingdom. Dramatic declines in populations in the UK and along the European coast were reported by Bryan et al. (1987). By 1991, imposex had been reported in 72 species of gastropods in 49 genera worldwide (Fioroni et al., 1991; Oehlmann et al., 1991). Five years later the number of species reported with imposex had risen to 120 (Oehlmann et al., 1996).

One of the first detections of imposex in Australia was made in 1991 at Rottneest Island, Western Australia (WA), 29 km offshore of Perth, the

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<http://dx.doi.org/10.1016/j.marpolbul.2017.08.064>

Received 3 May 2017; Received in revised form 23 August 2017; Accepted 25 August 2017
0025-326X/© 2017 Published by Elsevier Ltd.

only large population centre in the state. Kohn and Almasi (1993) reported imposex in six of the seven marine species of *Conus* (Mollusca: Caenogastropoda) they examined in 1991 (only a single specimen of the seventh species was collected). Overall 80% of the females examined had imposex. The most surprising aspect of this study was that most of the *Conus* were collected on three platforms at the western end of the island, where 88% of females demonstrated imposex, primarily at moderate Stages 3 and 4 on a scale of 0 to 6. The location is well offshore from the harbour city of Fremantle and away from shipping lanes. There is relatively little small boating activity and TBT concentrations would be expected to be the lowest in the Perth region. It is possible the site at Cape Vlamingh was contaminated by TBT from the wreck of a small commercial fishing vessel.

Imposex was rapidly reported in other parts of Australia; by 1993 it had been found in all mainland states, including in the marine gastropods *Nassarius* (Nassariidae) in Queensland (Mitchell, 1989), muricid *Dicathais orbita* (Gmelin, 1791) (as *Thais*) and ranellid *Cabestana spengleri* (Perry, 1811) in Victoria (Foale, 1993), muricid *Bedevea vinosa* (Lamarck, 1822) (as *Lepsiella*) in South Australia (Nias et al., 1993), and *D. orbita* and *Tenguela marginalba* (Blainville, 1832) (as *Morula*) in New South Wales (Wilson et al., 1993). There were also other reports of imposex in WA: *T. orbita* in Cockburn Sound and elsewhere (Field, 1993) and muricid *Tenguela granulata* (Duclos, 1832) (as *Morula*) in the Dampier Archipelago (Reitsema and Spickett, 1999). However, it was not recorded in *T. granulata* (as *Morula*) at the Rowley Shoals on the outer North-West Shelf, which had virtually no vessel traffic (Wells, 2000).

TBT has also been detected in the tissues of the Sydney rock oyster *Saccostrea glomerata* (Gould, 1850) (as *S. commercialis* (Iredale and Roughley, 1933)), where it causes abnormalities in shell growth (Batley et al., 1989), and intersex in abalone (*Haliotis roei* Gray, 1826) in WA (Sloan and Gagnon, 2004).

The WA Environmental Protection Authority (EPA) surveyed TBT in the Perth metropolitan area in 1991. The highest levels occurred in southern Cockburn Sound, a major shipping area, where 1350 µg per litre was recorded and at Careening Bay on Garden Island, with 1560 µg per litre (Fig. 1). In general, TBT was highest in small boat harbours and major shipping areas (Cary et al., 1991; Burt et al., 1993). In contrast, concentrations at the west end of Rottnest Island, where Kohn and Almasi (1993) collected most of their specimens, were < 10 µg per litre. Field (1993) subsequently made a more intensive survey of imposex stages in *D. orbita* in the Perth metropolitan area and found that up to 100% of the snails in small boat harbours were affected by imposex. Imposex levels decreased with distance from TBT sources.

In late 1991, the EPA banned the use of TBT in vessels < 25 m in length. TBT could not be prohibited on larger vessels as they are subject to regulation by the International Maritime Organization (IMO). Kohn et al. (1999) resurveyed *Conus* at Rottnest Island in January 1996 to determine the effectiveness of the limited TBT bans. A significant decrease was found in imposex in *C. dorreensis* Péron, 1807, the most common species, from 86% of females to 65%. The frequency also decreased in *C. sponsalis* Hwass in Bruguière, 1792, but increased in *C. klemae* (Cotton, 1953), but the changes were not statistically significant in the latter two species because of the limited numbers of snails that could be found. In both surveys, the most common stage of imposex was 3, a moderate degree of imposex (Kohn and Almasi, 1993; Kohn et al., 1999).

In 2001, the IMO adopted the *International Convention on the Control of Harmful Anti-Fouling Systems on Ships* but the required number of countries adopting the convention with the necessary percentage of world shipping tonnage was not reached until 2008; the convention came into force on 17 September 2008. From this date, organotin compounds could no longer be used as antifoulants in newly built vessels or existing vessels during heavy maintenance, including renewal of the antifouling coating, every five years. This provided a five year phase out period for TBT that ended in September 2013.

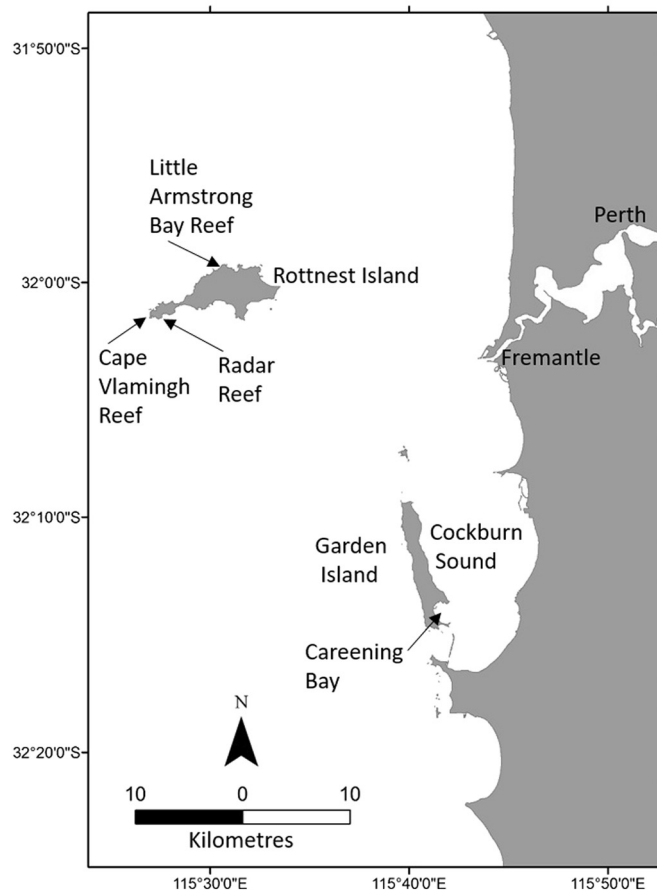


Fig. 1. Map showing the location of sample sites at Rottnest Island, Western Australia and the naval base on Cockburn Sound.

The present study resurveyed imposex in the same species and at the sites used by Kohn et al. (1999) in December 2007, prior to the IMO convention being adopted, and in February 2017, 3.5 years after the last use of TBT permitted under the antifouling convention. The goals were to determine the present TBT levels in *Conus* and to provide a baseline for monitoring any future imposex caused by copper, which is now being used as the antifoulant.

2. Methods

Gibbs et al. (1987) introduced two methods for measuring the severity of imposex: relative penis size (RPS) and vas deferens sequence (VDS). Both have subsequently been used, but most studies have used the VDS. The VDS was used in the present study as modified by Fioroni et al. (1991) and Oehlmann et al. (1991, 1996) to be consistent with its use in the earlier Rottnest Island studies (Kohn and Almasi, 1993; Kohn et al., 1999). The VDS analyses anatomical changes in the snails as imposex becomes increasingly severe. As the anatomical changes are irreversible in the individual they provide a permanent record of exposure to TBT and severity. The anatomical modifications are independent of seasonality or preservation artefacts (Oehlmann et al., 1996). The stages of the VDS are:

- 0 : normal female without any male sex characters
- 1 a: primordial penis but no penis duct or vas deferens. 1 b: short distal vas deferens only. 1c: short vas deferens only at the bottom of the mantle cavity.
- 2 a: penis with closed penis duct but no vas deferens. 2b: proximal and distal segments of vas deferens but no penis. 2c: penis with no duct

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